SciMedCentral

Annals of Marine Biology and Research

Research Article

Arsenic Induced Inflammation and Apoptosis in Liver, Head-Kidney and Skin of Gilthead Seabream (*Sparus aurata*)

Cordero H, Guardiola FA, Cuesta A, Meseguer J, and Esteban MA*

Department of Cell Biology and Histology, University of Murcia, Spain

***Corresponding author**

Esteban MA, Department of Cell Biology and Histology, Faculty of Biology, University of Murcia. 30100 Murcia, Spain, Tel: 34-868887665; Fax: 34-868883963; E-mail: aesteban@um.es

Submitted: 31 October 2014

Accepted: 09 November 2014

Published: 11 November 2014

Keywords

- Arsenic
- Inflammation
- Apoptosis
- Gilthead seabream (*Sparus aurata* L.)

Abstract

Arsenic is a metal with strong impact on the aquatic environment but its effects on marine fish immunity is little known. In this study, we have evaluated the regulation in the expression of genes encoding important acute-phase proteins (ceruloplasmin, transferrin and vitellogenin), antimicrobial peptides (β-defensin, hepcidin and histone 2B) and apoptosis (caspases 3, 8 and 9) in the skin, liver and head-kidney (HK) of the marine gilthead seabream (*Sparus aurata* L.) after waterborne As acute exposure for 2 or 10 days. Ceruloplasmin and vitellogenin transcription was significantly increased at 2 days on skin whilst the transferrin gene was down-regulated in HK and skin after 2 and 10 days, respectively. Concerning the antimicrobial peptides, β-defensin and hepcidin were firstly up-regulated in the HK and later they were up-regulated in the skin as well as the histone 2B gene. Finally, apoptosis was induced in all the tissues after As-exposure as indicated by the up-regulation of caspase genes. The data obtained provide an approach for elucidating the different molecular mechanisms induced by arsenic toxicity in marine fish.

ABBREVIATIONS

As: Arsenic; APPs: Acute-Phase Proteins; AMPs: Antimicrobial Peptides; bd: Beta-Defensin; Cd: Cadmium; cDNA: Complementary Deoxyribonucleic Acid; Cp: Ceruloplasmin; casp: Caspase; ef1a: Elongation Factor 1-Alpha; hamp: Hepcidin; h2b: Histone 2b; HK: Head-Kidney; mRNA: Messenger Ribonucleic Acid; PCR: Polymerase Chain Reaction; Tf: Transferrin; Vg: Vitellogenin.

INTRODUCTION

Arsenic (As) is a naturally occurring element found in soil, air and water [1,2]. The most toxicologically potent As compounds are inorganic and concretely in the trivalent oxidation state [3]. Most studies to understand the toxicity of As compounds were performed in mammals and is associated with liver, lung and skin cancers in humans [4]. In addition, As induces oxidative stress [5] and apoptosis [6] among other effects. However, less is known about other organisms and, for example, very little is known about the As toxicity in fish biology. The *in vitro* mechanism of As induced toxicity in fish cell lines (oxidative stress, disruption of mitochondrial potential, apoptosis, etc.) is similar to that observed in mammalian cell lines [7]. In addition, *in vivo* studies reveal that As caused different histopathological alterations in several tissues as well as alterations of the immune response in fish [8–11]. Focusing on the fish immune response, the scarce available information points to macrophages as the main targets for As-toxicity [12,13]. However, other fish immune aspects have been ignored. For example, acute-phase proteins (APPs) are a key component of the innate immune system in teleosts and used as inflammation indicators [14]. In this way, among them, transferrin (Tf) and ceruloplasmin (Cp) are vital [15], whilst others such as vitellogenin (Vg) have recently been suggested with a potential role as acute-phase proteins and microbial peptides [16,17]. During the last years, study of the abundance and role of fish antimicrobial peptides (AMPs) has attracted the interest of researchers due to the great role they play in the fish innate immune response, and they can be perturbed by As [8].

Since very little is known about the specific effects of As at molecular levels on fish, this paper describes, for the first time, inflammatory and apoptotic effects of waterborne As-exposure in the expression of different genes in the liver, head-kidney and skin of gilthead seabream (*Sparus aurata*). Selected genes were grouped into three categories: acute-phase proteins (ceruloplasmin (*cp*), transferrin (*tf*) and vitellogenin (*vg*)), antimicrobial peptides (β-defensin (*bd*), hepcidin (*hamp*) and histone H2B (*h2b*)) and apoptosis cell-death (caspase-3, -8 and -9 (*casp3*, *8* and *9*)], all relating to the immunity. We aimed to evaluate if the presence of As into the water is capable to modulate gene expression in the gilthead seabream, especially in inflammatory and apoptotic processes.

MATERIALS AND METHODS

Fish, arsenic exposure and sampling

Twenty-four specimens (41.5 ± 18.1 g body weight and 13.7 ± 2.6 cm body-length) of gilthead seabream (*Sparus aurata* L.), obtained from Doramenor Acuicultura S.L. (Murcia, Spain), were kept in two separate seawater aquaria (250 L). The water was maintained at 20 ± 2°C with a flow rate of 900 l h^{-1} in closed recirculating system, and 28‰ salinity. The photoperiod was of 12 h light: 12 h dark and fish fed with a commercial pellet diet (Skretting, Spain) at a rate of 2% body weight day^{-1}.

Fish were unexposed (control) or exposed to waterborne arsenic by adding 5μM As_2O_3 (Fluka Analytical) into the tank water. Six fish per tank and group were sampled after 2 and 10 days of exposition. Fish were starved for 24 h prior to sampling and sacrificed by an overdose of MS222 (Sandoz, Spain, 100 mg ml^{-1} water) [18]. Tissue fragments of skin; liver and head-kidney (HK) were obtained and immediately frozen in TRIzol Reagent (Life Technologies) for later RNA isolation. All experimental protocols were approved by the Bioethical Committee of the University of Murcia.

Real-time PCR

Relative gene expression was analysed in six fish per treatment using real-time PCR and the $2^{-\Delta\Delta CT}$ method [19]. Liver, head-kidney and skin RNA was extracted with TRIzol reagent (Life Technologies) following manufacturer's instructions, quantified and the purity assessed by spectrophotometry; the 260:280 ratios were 1.8-2.0. The RNA was then treated with DNase I (Promega) to remove genomic DNA contamination. Complementary DNA (cDNA) was synthesized from 1 μg of total mRNA using the SuperScript III reverse transcriptase (Life Technologies) with an oligo-dT18 primer. The expression of ten selected genes was analyzed by real-time PCR, which was performed with an ABI PRISM 7500 instrument (Applied Biosystems) using SYBR Green PCR Core Reagents (Applied Biosystems). Reaction mixtures (containing 10 μl of 2xSYBR Green supermix, 5 μl of primers (0.6 μM each) and 5 μl of cDNA template) were incubated for 10 min at 95ºC, followed by 40 cycles of 15 s at 95ºC, 1 min at 60ºC, and finally 15 s at 95ºC, 1 min at 60ºC and 15 s at 95ºC. For each mRNA, gene expression was corrected by the elongation factor 1-alpha (*ef1a*) content in each sample. The primers used are shown in (Table 1) and every product size is between 90 and 120 bp according to the design of the primers. In all cases, each PCR was performed with triplicate samples.

Statistical analysis

Data are expressed as fold increase (mean ± standard error, SE), obtained by dividing each sample value by the mean control value at the same sampling time. Values higher than 1 express an increase while values lower than 1 express a decrease in the indicated gene. Data were statistically analysed by the t-Student test using SPSS 19 to determine differences between unexposed and exposed groups. Differences were considered statistically significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

The expression of the genes encoding positive acute-phase proteins, *cp* and *vg* [15], was significantly increased in skin and remained unaltered in head-kidney and liver in fish exposed to As for 2 days (Figure 1). Nevertheless, the putative APP, *tf* [16,17], gene expression was significantly decreased in HK and skin after 2 and 10 days of exposure, respectively. The most significant changes were found in skin, whilst no significant changes were observed in the expression of APP genes in the liver from As-exposed fish compared to gene expression in control specimens (Figure 1).

Regarding genes encoding AMPs (*bd, hamp* and *h2b*), a non-significant increase was found in liver whereas *bd* and *hamp* expression showed a significant increase in HK after 2 days of As exposure (Figure 2). However, all the AMP genes were up-

Table 1: Oligonucleotide primers used for real-time PCR.

Gene	Abbreviation	GenBank ID	Primer sequence (5 → 3)
Elongation factor 1-alpha	*ef1a*	AF184170	F: CTGTCAAGGAAATCCGTCGT R: TGACCTGAGCGTTGAAGTTG
Ceruloplasmin	*cp*	FM145678	F: GATAAGGCGGGTGGGTTTAT R: GGTGTTCCATATCGGCTGTT
Transferrin	*tf*	FM158209	F: CAGGACCAGCAGACCAAGTT R: TGGTGGAGTCCTTGAAGAGG
Vitellogenin	*vg*	JF309047	F: ATGTCGACCTGTACCCCAAG R: TTGCCTGCAGGATGATGATA
β-defensin	*bd*	AF210428	F: CCCCAGTCTGAGTGGAGTGT R: AATGAGACACGCAGCACAAG
Hepcidin	*hamp*	CB184616	F: GCCATCGTGCTCACCTTTAT R: CTGTTGCCATACCCCATCTT
Histone H2B	*h2b*	AM953480	F: AGACGGTCAAAGCACCAAAG R: AGTTCATGATGCCCATAGCC
Caspase 8	*casp8*	AM975716	F: GAACAGGGAGGTCAGCAAAG R: ACACACGCTTGAGGACGAG
Caspase 9	*casp9*	AM959613	F: ATAGTTGCCGTCTCGCTCAC R: TCACGAGATTGCTCCTGTTG
Caspase 3	*casp3*	EU722334	F: CTGATCTGGATGGAGGCATT R: AGTAGTAGCCTGGGGCTGTG

regulated in the skin at 10 days of fish exposition being the greatest differences found for bd and *hamp* (Figure 2).

Finally, the expression of genes involved in apoptosis cell death seems to play a special role in As toxicity. Two different initiator apoptosis caspase genes (*casp8* and *casp9*) and an executioner apoptotic caspase (*casp3*) were analyzed. Initiator *casp8* gene expression showed a significant increase at 2 days in liver and HK, whilst initiator *casp9* gene expression was up-regulated in skin of As-exposed fish (Figure 3). *casp9* and *casp3* transcription was down-regulated at 2 days of As exposure in liver and skin, respectively. Strikingly, executioner apoptosis *casp3* expression was statistically significant up-regulated in liver, HK and skin after 10 days of exposure (Figure 3).

Previous results obtained after As-exposure in the gilthead seabream demonstrated histopathological and immunotoxicological effects including increase of hepato-somatic index, liver bioaccumulation, inflammation and cellular vacuolization and apoptotic processes as well as increase in the phagocyte-related immune function in the HK leucocytes [13]. All this led us to investigate the expression of genes related to aspects such as inflammation, immune defence and apoptosis in the liver and HK of gilthead seabream exposed to As. In addition, the skin was chosen in the present work because this organ is an essential protective barrier in innate immune system of fish [20] and the most exposed, and first, to waterborne As.

Therefore, different genes were selected to verify the immune status and apoptotic processes at molecular level in *Sparus aurata*. We selected the acute-phase protein genes *cp*, *tf* and *vg* as inflammatory and stress indicators for the presence of As in the water, synthesized in liver and expressed in fish HK [14] and also in fish skin [21]. Three antimicrobial peptide genes (*bd*, *hamp* and *h2b*) were selected as innate immune system indicators [22]. To our knowledge, these two aspects have never been evaluated in fish exposed to As. Furthermore, it has been demonstrated that As promotes apoptosis in fish both *in vitro* [7] and *in vivo* [23]. Taking this into account, we chose three genes involved in the apoptotic pathway, two initiator caspase genes (*casp8* and *casp9*), and an executioner apoptotic caspase gene (*casp3*) [24].

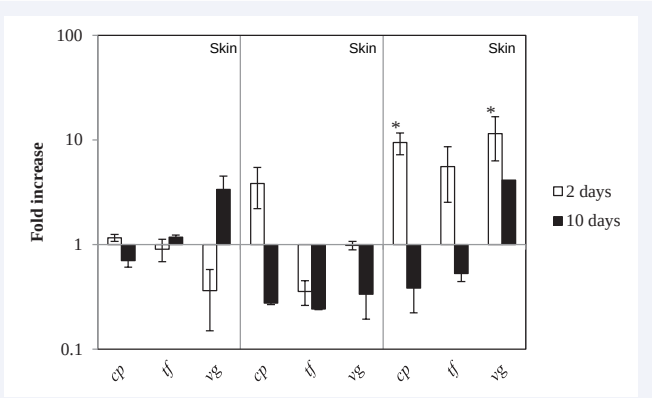

Figure 1 Expression of genes encoding acute-phase proteins (ceruloplasmin, *cp*; transferrin, *tf*; and vitellogenin, *vg*) determined by real-time PCR in liver, head-kidney and skin of gilthead seabream after 2 (white bars) and 10 (black bars) days of waterborne exposure to 5 µM arsenic. The bars represent the means ± SEM (n=6) fold increase relative to control. Asterisks denote significant differences when p≤0.05 between unexposed and As-exposed groups.

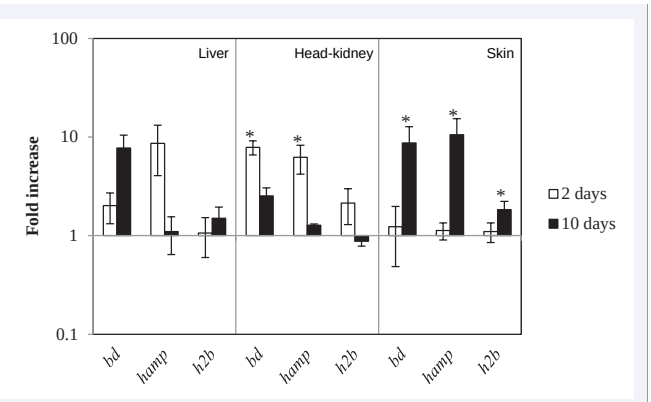

Figure 2 Expression of genes encoding antimicrobial peptides (beta-defensin, *bd*; hepcidin, *hamp*; and histone H2B, *h2b*) determined by real-time PCR in liver, head-kidney and skin of gilthead seabream after 2 (white bars) and 10 (black bars) days of waterborne exposure to 5 µM arsenic. The bars represent the means ± SEM (n=6) fold increase relative to control. Asterisks denote significant differences when p≤0.05 between unexposed and As-exposed groups.

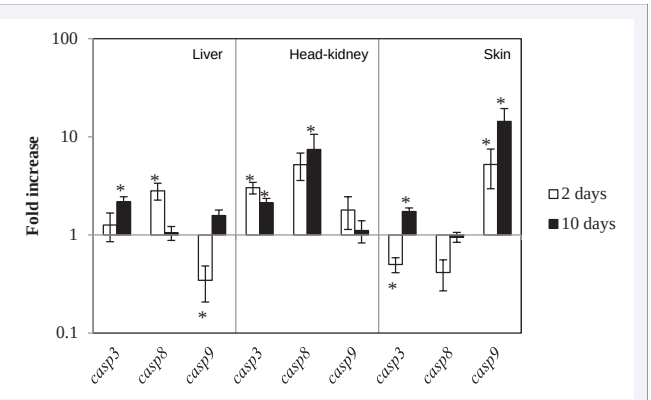

Figure 3 Expression of genes involved in apoptosis (caspase-8, *casp8*; caspase-9, *casp9*; and caspase-3, *casp3*) determined by real-time PCR in liver, head-kidney and skin of gilthead seabream after 2 (white bars) and 10 (black bars) days of waterborne exposure to 5 µM arsenic. The bars represent the means ± SEM (n=6) fold increase relative to control. Asterisks denote significant differences when p≤0.05 between unexposed and As-exposed groups.

Regarding acute phase response, it was reported that As induced the expression of stress-related genes [5]. Transferrin is considered a negative acute protein in mammals [14]. However, in other study, it was demonstrated that is a positive acute phase protein [21,25] and antimicrobial capacity has also been associated to transferrin in fish [26]. Moreover, transferrin and ceruloplasmin are two of the most important APPs [15,27] which act on iron homeostasis processes in order to increase iron storage to make it unavailable for bacterial growth [28]. Our results in *tf* and *cp* gene expression showed no changes in liver but significantly increased in skin after 2 days of As-exposure, suggesting that it may be due to skin inflammation and/or stress. This also occurs with *vg* which is also considered an APP [16] and is expressed in fish skin [21]. All these data demonstrate

that acute As-exposure increases APP gene expression mainly in gilthead seabream skin suggesting inflammation in the most exposed tissue.

Discover of the AMP function in the immune response include the most typical of direct lytic activity against pathogens, but also other important functions such as mediators of inflammation and its modulation [29]. It is well-known that β-defensin is an AMP gene synthesized in HK leucocytes and mostly expressed in fish skin, including the seabream [30]. According to this work, our results show up-regulation in HK after 2 days of As-exposure, which could be indicative of its synthesis by As-activated phagocytes [13] and later recruitment of these cells to the skin, the tissue in which AMP genes are overexpressed after 10 days. Interestingly, *hamp* and *h2b*, also considered AMP in fish [29,31] are up-regulated at the same time of As-exposition in HK and skin, respectively. These findings could indicate that As-induced expression of AMP genes in the skin could be due to the recruitment of phagocytes from the head-kidney but also the up-regulation in skin-resident leucocytes since *bd* and *hamp* genes are highly expressed in the skin of naïve seabream specimens [29,30]. Moreover, it has been recently demonstrated that As-exposure causes loss of gap junction [32], which could alter the permeability in the skin barrier of fish allowing pathogen entry with the consequent immune response though new molecular and cellular studies are needed to confirm this hypothesis in fish.

Last but not least, apoptosis or programmed cell-death caspase-dependent pathway is executed by caspase-3 after stress cell or damage [33]. Regarding fish, it was reported that As induces apoptosis in liver and HK macrophages of catfish [23,34]. Based on these data and considering previous studies at cellular level in *Sparus aurata* [13], we analysed *casp8*, *casp9* and *casp3* gene expression in liver, HK and skin. Interestingly, only executioner apoptotic caspase, *casp3*, was up-regulated after 10 days of As-exposure in the three organs. A recent research demonstrated that Cd-exposure induces apoptosis through caspase-3 activation in red common carp [35]. Moreover, specifically with As-exposure, liver and HK apoptotic cells were detected by the caspase-3 activation pathway [10,23], as occurred in the present study. However, apoptotic processes in fish skin are poorly understood [36], and these results suggest an apoptotic effect by caspase-dependent pathway due to up-regulation of *casp3* expression after 10 days of As-exposure, although further research is needed to conclude the apoptotic molecular pathway induced by As-exposure.

CONCLUSION

The present study reveals alterations in the expression of genes related to acute-phase proteins, antimicrobial peptides and apoptosis cell death after As-exposure in the gilthead seabream, especially in the skin, suggesting inflammation and cell death. Moreover, this research describes the potential function of the skin as an important source of APPs and AMPs. Last, apoptotic processes observed at cellular level in liver by previous studies [13] are now also confirmed at genetic level. These data throw some light into the toxicological mechanisms involved in the As toxicity in fish and reveal the importance of the skin, aspect that has never been considered before in fish.

FUNDING ACKNOWLEDGEMENTS

H. Cordero wishes to thank the *Ministerio de Economía y Competitividad* for a F.P.I. fellowship. This work was co-funded by a national project of the *Ministerio de Economía y Competitividad* (AGL2011-30381-C03-01) and *Fundación Séneca de la Región de Murcia (Grupo de Excelencia* 04538/GERM/06).

REFERENCES

1. Huang C, Ke Q, Costa M, Shi X. Molecular mechanisms of arsenic carcinogenesis. Mol Cell Biochem. 2004; 255: 57-66.
2. Duker AA, Carranza EJ, Hale M. Arsenic geochemistry and health. Environ Int. 2005; 31: 631-641.
3. Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ. Arsenic exposure and toxicology: a historical perspective. Toxicol Sci. 2011; 123: 305-332.
4. Lee CH, Liao WT, Yu HS. Aberrant immune responses in arsenical skin cancers. Kaohsiung J Med Sci. 2011; 27: 396-401.
5. Li M, Cai JF, Chiu JF. Arsenic induces oxidative stress and activates stress gene expressions in cultured lung epithelial cells. J Cell Biochem. 2002; 87: 29-38.
6. Ray A, Chatterjee S, Mukherjee S, Bhattacharya S. Arsenic trioxide induced indirect and direct inhibition of glutathione reductase leads to apoptosis in rat hepatocytes. Biometals. 2014; 27: 483-494.
7. Selvaraj V, Armistead MY, Cohenford M, Murray E. Arsenic trioxide (As(2)O(3)) induces apoptosis and necrosis mediated cell death through mitochondrial membrane potential damage and elevated production of reactive oxygen species in PLHC-1 fish cell line. Chemosphere. 2013; 90: 1201–1209.
8. Hermann AC, Kim CH. Effects of arsenic on zebrafish innate immune system. Mar Biotechnol (NY). 2005; 7: 494-505.
9. Ghosh D, Bhattacharya S, Mazumder S. Perturbations in the catfish immune responses by arsenic: organ and cell specific effects. Comp Biochem Physiol C Toxicol Pharmacol. 2006; 143: 455-463.
10. Datta S, Mazumder S, Ghosh D, Dey S, Bhattacharya S. Low concentration of arsenic could induce caspase-3 mediated head kidney macrophage apoptosis with JNK-p38 activation in Clarias batrachus. Toxicol Appl Pharmacol. 2009; 241: 329-338.
11. Pedlar RM, Ptashynski MD, Wautier KG, Evans RE, Baron CL, Klaverkamp JF. The accumulation, distribution, and toxicological effects of dietary arsenic exposure in lake whitefish (Coregonus clupeaformis) and lake trout (Salvelinus namaycush). Comp Biochem Physiol C Toxicol Pharmacol. 2002; 131: 73–91.
12. Ghosh D, Datta S, Bhattacharya S, Mazumder S. Long-term exposure to arsenic affects head kidney and impairs humoral immune responses of Clarias batrachus. Aquat Toxicol. 2007; 81: 79-89.
13. Guardiola FA, Gónzalez-Párraga MP, Cuesta A, Meseguer J, Martínez S, Martínez-Sánchez MJ, et al. Immunotoxicological effects of inorganic arsenic on gilthead seabream (Sparus aurata L.). Aquatic Toxicol. 2013; 134-135: 112–119.
14. Bayne CJ, Gerwick L. The acute phase response and innate immunity of fish. Dev Comp Immunol. 2001; 25: 725-743.
15. Peatman E, Baoprasertkul P, Terhune J, Xu P, Nandi S, Kucuktas H, et al. Expression analysis of the acute phase response in channel catfish (Ictalurus punctatus) after infection with a Gram-negative bacterium. Dev Comp Immunol. 2007; 31: 1183–1196.
16. Tong Z, Li L, Pawar R, Zhang S. Vitellogenin is an acute phase protein

with bacterial-binding and inhibiting activities. Immunobiology. 2010; 215: 898-902.

17. Shi X, Zhang S, Pang Q. Vitellogenin is a novel player in defense reactions. Fish Shellfish Immunol. 2006; 20: 769-772.

18. Esteban MA, Meseguer J. Phagocytic defence mechanism in sea bass (Dicentrarchus labrax L.): an ultrastructural study. Anat Rec. 1994; 240: 589-597.

19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25: 402-408.

20. Esteban MA. An Overview of the immunological defenses in fish skin. ISRN Immunology. 2012; 1–29.

21. Lü A, Hu X, Xue J, Zhu J, Wang Y, Zhou G. Gene expression profiling in the skin of zebrafish infected with Citrobacter freundii. Fish Shellfish Immunol. 2012; 32: 273-283.

22. Valero Y, Chaves-Pozo E, Meseguer J, Esteban MA, Cuesta A. Biological role of fish antimicrobial peptides. Antimicrobial Peptides. 2013; 2: 31-60.

23. Datta S, Saha DR, Ghosh D, Majumdar T, Bhattacharya S, Mazumder S. Sub-lethal concentration of arsenic interferes with the proliferation of hepatocytes and induces in vivo apoptosis in Clarias batrachus L. Comp Biochem Physiol C Toxicol Pharmacol. 2007; 145: 339-349.

24. Tait SW, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. Nat Rev Mol Cell Biol. 2010; 11: 621-632.

25. Audunsdottir SS, Magnadottir B, Gisladottir B, Jonsson ZO, Bragason BT. The acute phase response of cod (Gadus morhua L.): expression of immune response genes. Fish Shellfish Immunol. 2012; 32: 360–367.

26. Liu H, Takano T, Abernathy J, Wang S, Sha Z, Jiang Y. Structure and expression of transferrin gene of channel catfish, Ictalurus punctatus. Fish Shellfish Immunol. 2010; 28: 159-166.

27. Gitlin JD. Transcriptional regulation of ceruloplasmin gene expression during inflammation. J Biol Chem. 1988; 263: 6281-6287.

28. Liu H, Peatman E, Wang W, Abernathy J, Liu S, Kucuktas H. Molecular responses of ceruloplasmin to Edwardsiella ictaluri infection and iron overload in channel catfish (Ictalurus punctatus). Fish Shellfish Immunol. 2011; 30: 992-997.

29. Cuesta A, Meseguer J, Esteban MA. The antimicrobial peptide hepcidin exerts an important role in the innate immunity against bacteria in the bony fish gilthead seabream. Mol Immunol. 2008; 45: 2333-2342.

30. Cuesta A, Meseguer J, Esteban MÁ. Molecular and functional characterization of the gilthead seabream Î²-defensin demonstrate its chemotactic and antimicrobial activity. Mol Immunol. 2011; 48: 1432-1438.

31. Bergsson, G. Antimicrobial polypeptides and lipids as a part of innate defense mechanism of fish and human fetus. 2005.

32. Hsiao PJ, Jao JC, Tsai JL, Chang WT, Jeng KS, Kuo KK. Inorganic arsenic trioxide induces gap junction loss in association with the downregulation of connexin43 and E-cadherin in rat hepatic "stem-like" cells. Kaohsiung J Med Sci. 2014; 30: 57-67.

33. Creagh EM, Conroy H, Martin SJ. Caspase-activation pathways in apoptosis and immunity. Immunol Rev. 2003; 193: 10-21.

34. Banerjee C, Goswami R, Datta S, Rajagopal R, Mazumder S. Arsenic-induced alteration in intracellular calcium homeostasis induces head kidney macrophage apoptosis involving the activation of calpain-2 and ERK in Clarias batrachus. Toxicol App Pharm. 2011; 256: 44–51.

35. Gao D, Xu Z, Qiao P, Liu S, Zhang L, He P. Cadmium induces liver cell apoptosis through caspase-3A activation in purse red common carp (Cyprinus carpio). PLoS One. 2013; 8: e83423.

36. Sugimoto M, Uchida N, Hatayama M. Apoptosis in skin pigment cells of the medaka, Oryzias latipes (Teleostei), during long-term chromatic adaptation: the role of sympathetic innervation. Cell Tissue Res. 2000; 301: 205-216.

Cite this article

Cordero H, Guardiola FA, Cuesta A, Meseguer J, Esteban MA (2014) Arsenic Induced Inflammation and Apoptosis in Liver, Head-Kidney and Skin of Gilthead Seabream (Sparus aurata). Ann Mar Biol Res 1(1): 1001.